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Article Title < >
Journal or Book Title <Biochem. J.:>
Volume And Issue if a Journal < 340>
Pages if a Journal < 837-843 >
Year Of Publication < 1999>

Author (if known) <Jin et al >
Article Title < >
Journal or Book Title <Biochem. Biophys. Res. Commun.>
Volume And Issue if a Journal < 194>
Pages if a Journal < 496-503>
Year Of Publication < 1993>

Author (if known) <Lazard et al>
Article Title < >
Journal or Book Title <Nature:>
Volume And Issue if a Journal < 349>
Pages if a Journal < 790-793 >
Year Of Publication < 1991>

Thanks,

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components, again either because of damage directly to modality-specific lexical representations or because of damage to access of these representations. The implication of this conclusion is that phonological and orthographic output representations are organized by grammatical category.

(3) Finally, the facts in (1) and (2) and the fact that H.W. and S.J.D. were impaired in processing only the verb form of homonymic words imply that the deficit does not concern specific lexical forms (for example, the orthographic form *crack*) but the grammatical category verb (of which *crack* is an instance) for modality-specific lexical forms. One implication of this latter conclusion is that we should give serious consideration to the possibility that grammatical category information is represented separately and redundantly in each modality-specific lexical system.

In summary, taken together with recent results of category-specific deficits, the results we have reported suggest a remarkably specific organization of lexical knowledge in the brain, both at the semantic[8] and at the lexical form levels. Although at this time we do not have clear hypotheses about the nature of the brain mechanisms that compute lexical structure, it is clear that the information computed by these mechanisms must represent not only the phonological and orthographic form of words but also their grammatical class. The results reported for H.W. and S.J.D. pose a serious challenge for those models of lexical processing that would dispense with linguistic level information in the representation of lexical knowledge[29]. □

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Odorant signal termination by olfactory UDP glucuronosyl transferase

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THE onset of olfactory transduction has been extensively studied[1–7], but considerably less is known about the molecular basis of olfactory signal termination[6,8,9]. It has been suggested that the highly active cytochrome P_{450} monooxygenases of olfactory neuroepithelium[10–12] are termination enzymes[5,8,11,12], a notion supported by the identification and molecular cloning of olfactory-specific cytochrome P_{450}s (refs. 13–16). But as reactions catalysed by cytochrome P_{450} (refs 17, 18) often do not significantly alter volatility, lipophilicity or odour properties[9,11], cytochrome P_{450} may not be solely responsible for olfactory signal termination. In liver and other tissues, drug hydroxylation by cytochrome P_{450} is frequently followed by phase II biotransformation, for example by UDP glucuronosyl transferase (UGT), resulting in a major change of solubility and chemical properties[19]. We report here the molecular cloning and expression of an olfactory-specific UGT. The olfactory enzyme, but not the one in liver microsomes, shows preference for odorants over standard UGT substrates. Furthermore, glucuronic acid conjugation abolishes the ability of odorants[1,20] to stimulate olfactory adenylyl cyclase. This, together with the known broad spectrum of drug-detoxification enzymes[17,19], supports a role for olfactory UGT in terminating diverse odorant signals.

We previously obtained peptide sequences of bovine gp56, an olfactory epithelial-specific transmembrane glycoprotein which is similar to UGT enzymes[16]. Based on the sequence information, a partial bovine complementary DNA clone (no. 21) encoding this protein was isolated. Using clone 21 as a probe, we isolated a full-length rat olfactory UGT clone (RO1) from a rat olfactory cDNA library (Fig. 1). The UGT clone shares several structural features with other UGT sequences (Fig. 1*b*). It is 87% identical to the partial bovine sequence (a possible orthologue), but only 44–60% identical to other rat liver UGT sequences (Fig. 1*b*). This, together with the expression results below, shows that a novel functional form, termed UGT_{olf}, has been identified which defines a new subfamily within the UGT superfamily[21].

As several olfactory signal-processing enzymes are tissue-specific[4,7,22], we examined the tissue specificity of clone RO1. Figure 2 shows that cross-hybridizing messenger RNA is exclusively expressed in olfactory epithelium, with no reactivity seen in liver or other UGT-containing tissues. A similar tissue specificity has been observed for protein gp56 using immunoblot analysis[16].

The tissue-specific expression of UGT_{olf} points to a possible role in olfaction. By analogy with UGT function in liver[19], olfactory UGT could help clear lipophilic odorant molecules from the chemosensory epithelium. We therefore asked whether olfactory UGT could glucuronidate odorants. When transiently expressed in COS-7 cells, rat UGT_{olf} catalysed the glucuronidation of numerous, chemically diverse substrates, with several odorants showing particularly high efficiency (Fig. 3*a*). In parallel, we measured the enzyme activity in microsomal membranes from olfactory epithelium. Several established[23] (hydroxyl-containing) odorants were efficient UGT substrates (10–70 nmol per mg protein per min) in preparations from cow[24] (Fig. 3*b*) and rat (data not shown). The activity profile of UGT_{olf} expressed in COS cells (Fig. 3*a*) is similar to that of the olfactory microsomal enzyme (Fig. 3*b*). Thus, UGT_{olf} is probably a dominant component of the overall UGT activity in the olfactory epithelium. For all five odorants shown, the olfactory enzyme was two- to fivefold more active than the liver enzyme. In contrast, glucuronation of classical UGT substrates was comparable in both tissues (Fig. 3*b*).

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To find out whether odorant glucuronidation could underlie olfactory signal termination, we compared the ability of odorants and their respective glucuronates to activate olfactory adenylyl cyclase, a reaction widely used as an *in vitro* assay for olfactory transduction activity[1-4,6]. Figure 3*c* shows that preincubation of odorants with olfactory microsomes under conditions leading to highly efficient glucuronidation results in a practically complete abolition of their ability to stimulate cyclic AMP generation. This does not occur if UDP-glucuronic acid or microsomes are omitted (Fig. 3*c*), or when heat-inactivated microsomes are used (data not shown).

Finally, UGT_{olf} was immunolocalized with an antiserum to a synthetic peptide. Labelling in bovine tissue (Fig. 4) and in rat (data not shown) was mainly in subepithelial Bowman's glands. Some labelling was seen in a superficial epithelial layer, probably corresponding to the apical cytoplasm regions of the secretory supporting cells[8]. Practically no immunoreactivity was observed in respiratory epithelium (not shown). Similar immunolabelling was previously observed for cytochrome P_{450} and cytochrome P_{450} reductase[25,26].

UGT_{olf} has several properties that make it an excellent candidate for a chemosensory signal termination enzyme. First, it can act on hydroxylated products of mixed function monooxygenases (such as cytochromes P_{450})[11-13,16], as well as on carboxylic acids, amines and thiols[19], allowing modification of a multitude of odorants. Second, its active site inside the endoplasmic reticulum lumen[19,27] can be easily reached by the lipophilic, membrane-penetrable odorants. The resulting membrane-impenetrable glucuronates may be secreted by vesicular traffic and removed by blood or mucus flow[8]. This hypothetical pathway is consistent with the localization of UGT_{olf} in secretory cells, and is similar to a mechanism suggested for the excretion of bilirubin glucuronates in the liver[28]. Third, in contrast to mere hydroxylation by cytochrome P_{450}, UGT-catalysed glucuronation results in a pronounced increase in hydrophilicity, preventing odorant repartition into cells. This will shorten tissue residence times and prevent continuous stimulation. Furthermore, as glucuronation abolishes the ability of odorants to activate adenylyl cyclase, odorants secreted into the mucus bathing the sensory cilia will not interfere with receptor responses to new stimuli. Finally, the high UGT activity in olfactory epithelial membranes (along with the high concentration of cytochrome P_{450} and NADPH-dependent cytochrome P_{450} reductase activity[11-13,25,26]) may compensate for possible slow kinetics[29], potentially allowing odorant removal within 0.1–10 seconds[8,9].

In addition to a role in rapid signal termination, olfactory

b

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                    --------------------                           ###
r-UGTolf    1     MLKNILLWSLQLSLLGMSLGGNVLIWPMEGSHWLNVKIIIDELLRKEHNVTVLVASGALFITPSVSPSLTFEIYPVPFGKEKIESVIKDFVLTWLENRPSPSTIWTF  107
c-UGTolf    1                                                                                          .S.G.....I...M.H....L...R.   26
UDPGTr-2    1  MSMKQTSVF.LIQLICYFRPGAC.K..V..T.Y...I.I...LN..AQRG.E.....S.ASIL.E.TKES.IN....S..LS.SDL.YSFAKWIDE.TRDFETL.-...Y  109
UDPGTr-4    1  MPRKWISA.FLLQISYCFKSGHC.K..V...DF...M.I...L...VQRG.E....KP.AYF.LD.KK.SD.K...FSTSIS.DELQNHFIKLLDV.TYEL.-RD.CLSY  109

r-UGTolf  108  YKEMAKVIEEFHLVSRGICDGVLKNEKLMTKLQRGKFEVLLSDPVFPCGDIVALKLGIPFIYSLRFSPASTVEKHCGKVPFPPSYVPAILSELTDQMSFADRVRNFISYP  217
c-UGTolf   27  .Q......KD...M...E...S..Q.....E..KKS.....V.......................................................T..I.......H  135
UDPGTr-2  110  .SK.Q..FN.YSD.VENL.KALIW.KS..K...GSQ.D.I.A.A.G...ELL.EL.KT.LV.....C.GYRC..FS.GL.L......VV....S.R.T.VE..K.MLQML  219
UDPGTr-4  110  SPILQNLV...SYFYLS..KDAVS.KQ......ES..D..FA...AS...LI.EL.H...L...S...GHKL..SI..FIL......V...G.AGK.T.I...K.M.CML  219

                                                                                                       ###
r-UGTolf  218  MQDYMFETLW-KQWDSYYSKALGRPTTLCETMGKAEIWLMRTYWDFEFPRPYLPNFEFVGGLHCKPAKPLPKEMEEFVQTSGEHGVVVFSLGSMVKNLTEEKANLIASAL  326
c-UGTolf  137  L.....N.....S.............................I.......................................S...D.I..............R.....  245
UDPGTr-2  220  YF.FW.QPFKE.S.SQF..DV.......T.M.....D...I..F..L...H.F....D................R.......S........................VV....  329
UDPGTr-4  220  YF.FW..R.RH.E..TF..EI.......VD...S.V....I.S...LK..H.T...VDYI...............D......S...................S.M......A..W..  329

r-UGTolf  327  AQIPQKVLWRYKGKIPATLGSNTRLFDWIPQNDLLGHPKTRAFITHGGTNGIYEAIYHGIPMVGVPMFADQPDNIAHMKAKGAAVEVNMNTMTSADLLSAVRAVINEPFY  436
c-UGTolf  246  ...............K.....A....Y.................AK.......................V..............................I.........N.L.T......S.  355
UDPGTr-2  330  .......V..FD..K.D.........YK.....................K..VA.......................I..I.L.........N..V.......R.DFSILSTTG..T.LKI.M.D.S.  439
UDPGTr-4  330  .........KFD..T.....P...VYK.L...................K..V.....A..L.....................I.L.G..............V.......SL.IR..SKI.F...LEE..EN..  439

                                                                           ++++++++++ + +++++
r-UGTolf  437  KENAMRLSRIHHDQPVKPLDRAVFWIEFVMRHKGAKHLRVAAHDLSWFQYHSLDVIGFLLACMASAILLVIKCCLFVFQKIGKTGKKNKKD  527
c-UGTolf  356  ......K.......................................P...N.T.Y...............V.T.VF..TR....SCR.F......K..E  446
UDPGTr-2  440  .............................Y..............STL..........................L.VVGVVFIIT.F...CCR.TANM...K.E  529
UDPGTr-4  440  .K.V.L..T......M..............I...........PLG.N.P.Y...............T.F.VIAA.TV..L..MYRFFV.KE..M.NE  530
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FIG. 1 Nucleotide and deduced amino-acid sequence of rat UGT_{olf}. *a* The nucleotide sequence of the rat UGT_{olf} (r-UGT_{olf}, clone R01) includes a 1,581-bp coding region (capitals) preceded by 63 bp non-coding sequence (lower case) and followed by 857 bp of non-coding sequence (lower case, 187 bp shown) ending in a polyadenylation signal (AATAAA) and a poly (A) stretch (not shown). The first ATG triplet in clone R01 defines the open reading frame of 527 amino acids (relative molecular weight 62,881), in close agreement with the length of other UGTs. *b*, Deduced amino-acid sequence of rat UGT_{olf} (r-UGT_{olf}, clone R01) and alignment[32] (gap weight, 5.0; length weight, 0.3, University of Wisconsin package) with the partial bovine UGT_{olf} (c-UGT_{olf}, clone 21) and two rat liver UGTs[33,34] (UDPGTr-2 and UDPGTr-4). Identities in the amino-acid sequences are indicated by dots. The following structural features are shared between UGT_{olf} and other known UGTs: (1) an N-terminal hydrophobic signal peptide (——); (2) two consensus sequences for *N*-linked glycosylation at position 49 and 313 (###), the latter conserved in several other UGTs; (3) a 17 residue long hydrophobic region near the carboxy-terminus (+++), most probably a single transmembrane anchor domain; (4) a cluster of positively-charged residues at the extreme carboxy terminus that appears to be a stop transfer signal; (5) conserved cysteines at positions 126, 157, 280, 498 and 510. The sequences of the three gp56 peptides previously reported[16] are underlined in the bovine sequence. Overall identities between the rat UGT_{olf} amino-acid sequence and other sequences are: bovine UGT_{olf}, 87%; UDPGTr-2, 60%; UDPGTr-4, 59%. The most conserved region is the C-terminal half of the protein. The highest diversity is found in the N-terminal 250 residues and in the 30-residue carboxy-terminal stretch. METHODS. A bovine olfactory epithelial cDNA library in λ-gt11 (Clontech, Palo Alto) was constructed using poly(A)$^{+}$-enriched RNA isolated from olfactory epithelium of three-month old calves. 350,000 plaques were screened with the following ^{32}P-labelled oligonucleotide mixtures (I is inosine): 5'-CCAIGT$^{A}/_{G}$TG$^{A}/_{G}$AA$^{A}/_{G}$TC$^{C}/_{T}$TTIATIAC$^{C}/_{T}$TTIG-CCAT-3' and 5'-TTIGC$^{C}/_{T}$TT$^{C}/_{T}$TC$^{C}/_{T}$TCIGTIA$^{A}/_{G}$IGT$^{C}/_{T}$TTIACCAT-3', corresponding to peptides 201 and 202 of gp56, respectively[16]. Three partial clones hybridizing with both oligonucleotides were purified and the 1,393-bp insert of one of them (14) was used for rescreening the cDNA library. This yielded a longer partial-length clone (21). Both clones included all the three reported gp56 peptides[16] in their deduced amino-acid sequence. A fragment of clone 21 (including most of the coding sequence) was used to screen a rat olfactory λgt11 cDNA library[13] with medium stringency washes (52 °C, 0.5 × SSC). Positive signals were observed at a frequency of 1 in 3,000. Fifteen clones were isolated and one (R01) subcloned in M13mp18 and sequenced on both strands of the coding region.

a

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   1  gaggactggaaagtagagaaatttgtttgttccacatttttagaagtttacatcaaacca
  61  gtcATGTTGAAGAACATTCTACTGTGGTCTCTTCAGCTAAGTCTCCTAGGAATGAGTCTT
 121  GGCGGGAATGTTCTCATCTGGCCAATGGAAGGCAGTCACTGGCTAAATGTTAAGATCATA
 181  ATAGACGAACTCCTAAGGAAAGAGCATAACGTGACTGTCCTTGTTGCATCCGGGGCCCTT
 241  TTCATCACACCGTCGGTCAGCCCATCTCTGACATTCGAAATATATCCAGTGCCCTTCGGC
 301  AAGGAAAAAATCGAAAGTGTGATTAAGGACTTTGTTTTGACGTGGCTGGAAAACAGGCCG
 361  TCTCCTTCAACTATCTGGACTTTCTATAAGGAAATGGCCAAAGTCATCGAGGAGTTCCAC
 421  TTGGTGTCCAGAGGGATCTGTGATGGCGTTCTTAAGAATGAAAAGCTGATGAGCAAGCTG
 481  CAGAGAGGAAAGTTTGAAGTACTGTTGTCAGACCCAGTATTCCCGTGTGGCGATATCGTA
 541  GCTTTAAAACTGGGGATTCCATTTATCTATTCCTTGCGGTTTTCTCCTGCCTCCACAGTG
 601  GAAAAGCACTGTGGAAAAGTCCCGTTTCCTCCTTCCTATGTTCCTGCAATCTTGTCAGAA
 661  CTCACAGACCAGATGTCCTTCGCTGACCGAGTAAGGAACTTTATCTCCTACCGAATGCAG
 721  GACTACATGTTTGAAACTCTTTGGAAGCAATGGGATTCCTATTACAGTAAAGCCTTAGGA
 781  AGACCCACCACACTGTGCGAGACTATGGGGAAAGCTGAGATTTGGCTAATGCGAACATAT
 841  TGGGATTTTGAATTTCCTCGTCCATATTTGCCTAATTTTGAGTTTGTGGGAGGACTGCAC
 901  TGCAAACCTGCTAAGCCTTTGCCTAAGGAAATGGAAGAGTTTGTTCAGACCTCAGGGGAA
 961  CATGGTGTTGTGGTGTTTTCTTTGGGGTCAATGGTCAAAAACCTGACAGAAGAAAAGGCC
1021  AATCTCATTGCCTCAGCCCTTGCCCAGATTCCTCAGAAGGTTTTGTGGCGATACAAAGGA
1081  AAGATACCAGCCACATTAGGATCCAATACGAGATTGTTTGACTGGATTCCTCAGAATGAT
1141  CTTCTTGGACATCCCAAAACCAGAGCTTTTATCACCCATGGTGGAACAAATGGAATCTAT
1201  GAGGCCATTTACCACGGGATCCCGATGGTGGGAGTTCCCATGTTCGCTGATCAGCCTGAC
1261  AACATAGCTCACATGAAGGCTAAAGGAGCAGCTGTGGAGGTGAACATGAACACGATGACG
1321  AGTGCAGATCTGCTCAGTGCTGTGAGAGCAGTCATCAATGAGCCATTTTATAAAGAGAAT
1381  GCCATGAGACTATCAAGAATTCACCACGATCAGCCAGTGAAGCCCCTGGACAGAGCCGTC
1441  TTCTGGATTGAGTTTGTCATGCGTCACAAAGGAGCCAAGCACCTTCGTGTGGCAGCGCAT
1501  GACCTCAGCTGGTTTCAGTACCACTCTCTAGATGTGATTGGGTTCCTGCTGGCTTGTATG
1561  GCATCTGCCATATTATTGGTTATAAAATGTTGTTTATTTGTATTTCAAAAAATTGGTAAG
1621  ACAGGAAAGAAGAATAAAAGGGACtagatgaaggaaagacatctcaggtctctcggcttgg
1681  gtcaagcaccaactggagtagcaatagtacttagaaacccagggagttgccagtcaccta
1741  tcctcatcccattttcaatcattccatt[illegible]ttagcttgagg[illegible]aaattggaggatcg
1801  aacatac[illegible]tttggctatttcaatcacaat
```

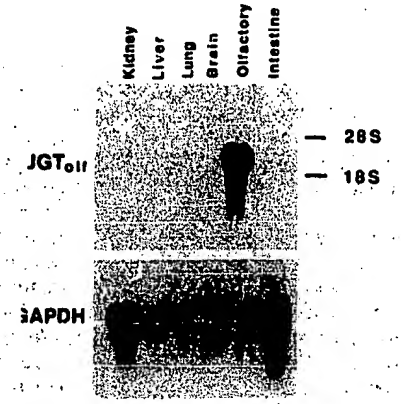
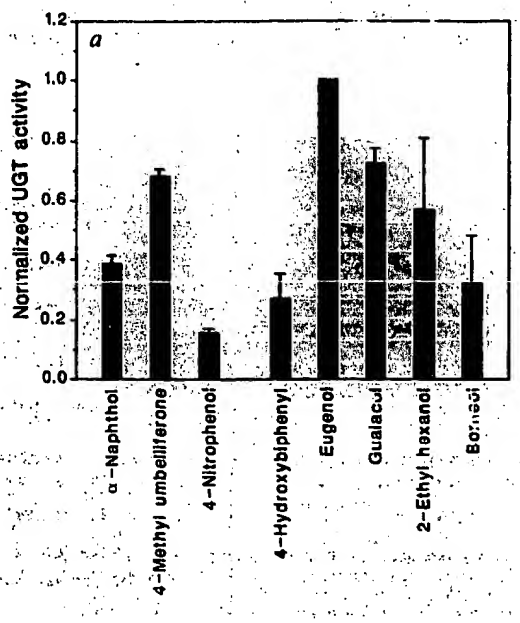


FIG. 2 Distribution of UGT_{olf} mRNA. Messenger RNAs from several rat tissues were probed with rat UGT_{olf}, and with a control glyceraldehyde phosphate dehydrogenase (GAPDH) probe. No detectable signal is seen in tissues other than olfactory epithelium, even with long exposure times. The apparent length of the mRNA is ~2.5 kb. A similar analysis for the bovine UGT clone (not shown) corroborates this observation, except that respiratory epithelium (an adjacent, non-sensory nasal tissue) is also positive, but has a 3–4-fold lower reactivity. Positions of 28S and 18S rRNA are marked.
METHODS. Rat tissues were dissected in the laboratory immediately after death and frozen in liquid nitrogen. Total RNA (20 μg) prepared as described[13] was electrophoresed on 1% agarose gels and blotted onto nylon membranes (Genescreen, DuPont). Membranes were hybridized with ^{32}P-labelled cDNA of clone RO1 (top) or mouse GAPDH (gift of Dr I. Ginsburg, the Weizmann Institute) (bottom). Hybridization was performed in Denhardt's solution with 1 M NaCl at 42 °C for 18 h, followed by two washes at 60 °C, 0.1 × SSC, for 30 min. Autoradiography was for 12 h at −70 °C.



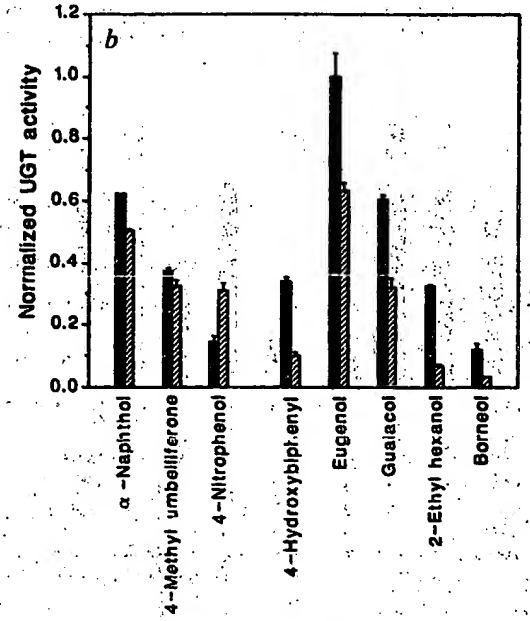


FIG. 3 Action and specificity of UGT_{olf}. *a*, expression of rat UGT_{olf} in COS-7 cells. UGT-activity towards standard substrates (three left-hand bars) and hydroxyl-containing odorants (five right-hand bars) was measured in homogenates of COS-7 cells transiently transfected with clone RO1 cDNA. No glucuronidation could be detected in membranes from untransfected cells or from cells transfected with the cDNA in the wrong orientation. Values are normalized relative to eugenol, and represent the mean ± difference of two experiments with two batches of transfected cells. Specific activities towards eugenol were 1.33 and 0.29 nmol per mg protein per min, and in untransfected cells were less than 0.008 nmol per mg protein per min. *b*, UGT activity towards the same substrates in bovine olfactory epithelial (dark) and liver (hatched) microsomes. Values (normalized as in *a*) are representative of two experiments, and each is the mean ± difference of two replicates. Specific activity towards eugenol was 72.7 ± 5.5 nmol per mg protein per min. *c*, Glucuronation abolishes odorant activation of olfactory adenylyl cyclase (mean ± s.d. of triplicates; $P < 0.02$). Odorants (or the vehicle alone for measurement of basal activity) were preincubated with or without bovine olfactory epithelial microsomes or UDP-glucuronic acid as indicated and subsequently used in the odorant-sensitive adenylyl cyclase assay[3]. ▨, Basal; ■, odorants.
METHODS. *a*, The RO1 cDNA was inserted in two possible orientations in the mammalian expression vector pSVL-RS (provided by Dr C. Kahana, the Weizmann Institute), a modified pSVL vector (Pharmacia) in which an *Eco*R1 site was introduced into the multiple cloning site. Subconfluent cultures of COS-7 cells were transfected with 0.24 μg recombinant plasmid per cm^2 as described[35,36]. After 72 h cells were ground-glass hand-homogenized and UGT activity assayed as described[37] with modifications: incubation was with 0.1 M Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 0.05% Triton X-100, 1 mM aglycone, 50 μM UDP-glucuronic acid, 0.1 μCi ^{14}C-labelled UDP-glucuronic acid (304 mCi mmol^{-1}; Dupont), and 10–60 μg homogenate protein in a total volume of 50 μl. After 2 h at 37 °C, the reaction was stopped with 150 μl ethanol, and samples analysed by thin-layer chromatography on silica gel as described[37]. *b*, Microsomes from bovine olfactory epithelium and liver were prepared as described[16]. UGT activity was assayed as described in *a*, except that the incubation mixture contained 1 mM UDP-glucuronic acid, 0.05 μCi ^{14}C-labelled UDP-glucuronic acid and 25 μg microsomal protein. Incubation time was 10 min and the reaction stopped with 3% SDS. Measurements were carried out in the excess substrate regimen, and glucuronates identified with β-glucuronidase[37]. *c*, A mixture of the four hydroxyl-containing odorants, eugenol, guaiacol, geraniol and 2-ethyl-1-hexanol (Aldrich, 62.5 μM each), was subjected to glucuronidation as in *b*, with the following modification: 100 μl reaction volume, 12 mM $MgCl_2$, no Triton X-100, 100 μg microsomal protein, 3 mM UDP-glucuronic acid, and 30 min incubation time, selected to maximize odorant glucuronidation. The reaction mixture was centrifuged (5 min, 12,000*g*) and 20 μl supernatant together with the adenylyl cyclase reaction mix and rat olfactory cilia (0.5–1.0 μg protein) were analysed for cAMP generation as described[1,38].

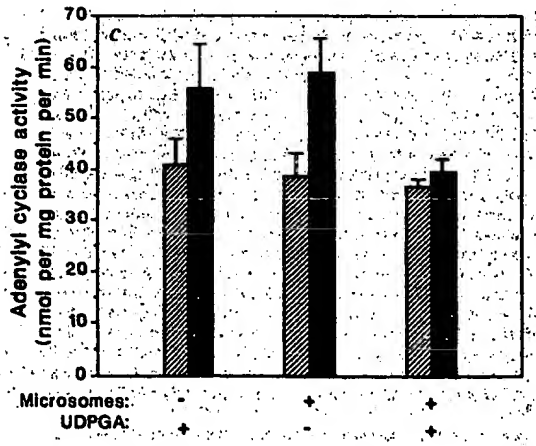


FIG. 4 Tissue localization of UGT_{olf}. Serial frozen sections of bovine olfactory epithelium were stained with *a*, haematoxylin-eosin or *b*, with an antiserum against UGT_{olf}. CIL, ciliary layer; SUP, supporting cells' apical cytoplasm and nuclei; NEU, sensory neuron nuclear layer; BAS, basal cell layer; SUB, subepithelial layers containing the Bowman's glands. *b*, Label is seen in the Bowman's glands (bg) and in a superficial layer (s). Labelling in the latter was more variable in location and intensity. No reactivity with olfactory epithelium was observed using non-specific rabbit serum, or an antiserum against an unrelated synthetic peptide, both purified as described below for the specific antiserum. The bar labelled SUP is 20 μm.

METHODS. Bovine olfactory epithelia were collected as in Fig. 3 and stored in isopentane at −80 °C. Sections (8–10 μm) were prepared on a Reichert–Jung Frigocut 2800 cryostat at −20 °C, dried overnight at 23 °C, fixed for 20 min in acetone at −20 °C and washed in sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl. Immunolabelling was performed with antiserum 11, prepared as described[16], against a BSA-conjugated synthetic peptide corresponding to residues 229–244 of bovine UGT_{olf}. An immunoglobulin fraction was obtained through DEAE-cellulose (Whatman) ion-exchange chromatography, and anti-BSA reactivity was removed by three passes of affinity chromatography on a BSA-Sepharose column (Pharmacia), following which radioimmunoassays for BSA were negative. Sections were incubated with concentrated purified antiserum 11 (0.4 mg protein ml^{-1}), and stained with tetramethylrhodamine-labelled goat anti-rabbit immunoglobulin antibody (gift of Dr B. Geiger, the Weizmann Institute).

cytochrome P_{450} and UGT may be long-term detoxification devices. Olfactory epithelium is the region of the nervous system most exposed to airborne hazardous chemicals, and is only a few millimetres from the brain. Olfactory epithelium has been proposed to be a direct route of pathological agents into the brain[30] and is related to early changes associated with Alzheimer's disease[31]. The study of olfactory cytochrome P_{450}s and UGT could shed light on a potentially relevant toxin inactivation mechanism. In cases where cytochrome P_{450} action leads to cytotoxin bioactivation[17,18], a highly active UGT may be the main detoxification barrier. □

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Activation of chloride channels in normal and cystic fibrosis airway epithelial cells by multifunctional calcium/calmodulin-dependent protein kinase

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Cystic fibrosis is associated with defective regulation of apical membrane chloride channels in airway epithelial cells. These channels in normal cells are activated by cyclic AMP-dependent protein kinase[1,2] and protein kinase C[3,4]. In cystic fibrosis these kinases fail to activate otherwise normal Cl^- channels[1–4]. But Cl^- flux in cystic fibrosis cells, as in normal cells, can be activated by raising intracellular Ca^{2+} (refs 5–10). We report here whole-cell patch clamp studies of normal and cystic fibrosis-derived airway epithelial cells showing that Cl^- channel activation by Ca^{2+} is mediated by multifunctional Ca^{2+}/calmodulin-dependent protein kinase. We find that intracellular application of activated kinase and ATP activates a Cl^- current similar to that activated by a Ca^{2+} ionophore, that peptide inhibitors of either the kinase or calmodulin block Ca^{2+}-dependent activation of Cl^- channels, and that a peptide inhibitor of protein kinase C does not block Ca^{2+}-dependent activation. Ca^{2+}/calmodulin activation of Cl^- channels presents a pathway with therapeutic potential for circumventing defective regulation of Cl^- channels in cystic fibrosis.

To delineate the molecular mechanism by which Ca^{2+} activates Cl^- channels, we used three simian virus 40 (SV40) transformed cell lines from normal and cystic fibrosis (CF) airway epithelia[3,11–13]. All three contain messenger RNA for the cystic fibrosis transmembrane regulator (CFTR)[14]. One of the CF cell lines (2CFSMEo-; CF-1) was positive for the ΔF508 mutation, the deletion in 70% of CF chromosomes[15], whereas the other (CFNPE-9o-; CF-2) was not[13].

Whole-cell patch clamp recording revealed that only the normal fetal cell line (56FHTE-8o-; refs 3, 20) showed increased Cl^- conductance in response to bath application of 400 μM

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